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(54) Title: ACYCLIC 6-PHENYLSELENENYL PYRIMIDINE NUCLEOSIDES

(57) Abstract

Acyclic 6-phenylselenenyl pyrimidine nucleosides with activity against HIV-1 and HIV-2. Antiviral compositions incorporating the novel compounds and methods of treatment employing such compositions are also disclosed.

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ACYCLIC 6-PHENYLSELENENYL PYRIMIDINE NUCLEOSIDES

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to analogues of acyclic pyrimidine nucleosides and antiviral compositions including the same.

2. Description of the Prior Art

Historically, efforts to discover effective antiviral agents have met with limited success. Since the viral and host cell replicative mechanisms were considered to be similar, it was difficult to envisage the inhibition of viral functions without causing unacceptable toxicity to the normal host cells. However, in recent years, it has become clear that most viruses, as they affect host cells, carry or produce unique enzymes which can be inhibited to achieve therapeutic specificity. The discovery and subsequent development of acyclovir to the point where it is now widely approved for clinical use against herpesvirus infections has confirmed this promise.

No one can claim a totally rational design for any of the active antiviral compounds, yet, it is clear that viruses code for several enzymes which are necessary for their replication, and that these enzymes appear to be less specific than the corresponding cellular enzymes in terms of their substrate specificity. This provides obvious targets against which to direct chemotherapeutic agents. As the normal

substrates for these enzymes are nucleoside derivatives, it is at least logical to expect that useful chemotherapeutic agents could be found among this class of compounds, and this has been shown to be the case.

With the human immunodeficiency virus (HIV), which is responsible for the symptoms of AIDS and ARC, the situation is similar to that which existed with the herpesvirus some ten years ago. At least one enzyme, the reverse transcriptase or RNA-dependent DNA polymerase, is unique to, and absolutely necessary for, viral replication. It should, therefore, be possible to target this enzyme for antiviral chemotherapy.

A number of synthetic and naturally occurring compounds which inhibit retroviruses have been identified. Some of these agents are undergoing clinical evaluations, and one drug, 3'-azido-3'-deoxythymidine (AZT) has been approved for treatment of AIDS patients. Unfortunately most of the anti-HIV agents identified so far are only virustatic and show inhibitory activity at or near toxic dose levels, which limits their usefulness. Furthermore, in the absence of an effective prophylactic compound, and due to the method of viral replication which results in integration of the viral coding capacity into the host genome, drug therapy following infection is likely to be required for long periods of time; thus, more effective and non-toxic compounds are required.

One class of compounds that have shown some promise as antiviral agents, including against HIV, are the 2',3'-dideoxy-nucleoside analogues.

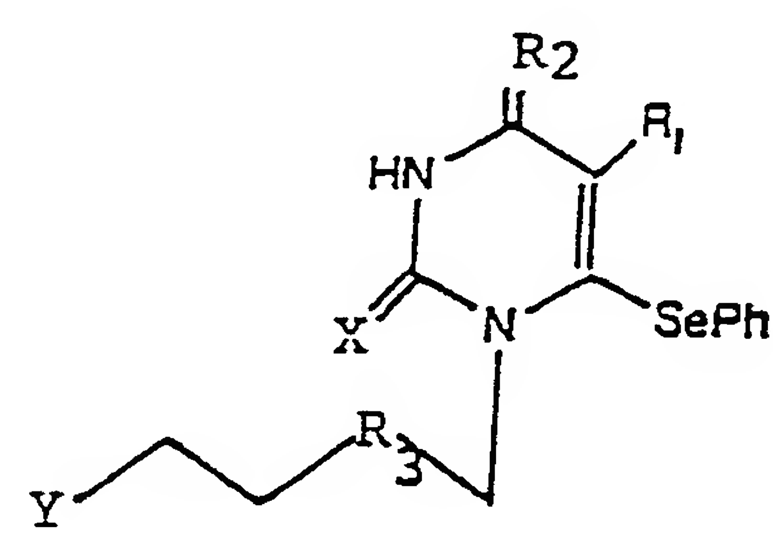
Extensive testing has been done with such compounds as 2',3'-dideoxyadenosine and dideoxycytidine, 3'-deoxythymidine and other dideoxy purine and pyrimidine nucleosides, as well as their 2',3'-didehydro analogues and substituted forms thereof. See generally C.K. Chu et al., Biochem. Pharm., 37:3543-3548 (1988); C.K. Chu et al., Tetrahedron Lett., 29:5349-5352 (1988); P. Herdewijn et al., J. Med. Chem, 30:1270-1278 (1987); V. Farina et al., Tetrahedron Lett., 29:1239-1242 (1988). Phosphate-linked nucleoside dimers of dideoxynucleosides and AZT have also been prepared and found to suppress HIV expression in vitro. Busso et al., AIDS Res. Hum. Retrovir., 4:449-455 (1988).

Recently, it was reported that 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine [HEPT-1] and 1-[(hydroxyethoxy)methyl]-6-(phenylthio)-2-thiothymine [HEPT-S 2] are potent and selective inhibitors of HIV-1, but not human immunodeficiency virus type 2 (HIV-2), in various human lymphocytes. In contrast to the current antiviral agents with a modified carbohydrate moiety, these 6-substituted compounds are acyclic nucleosides related to acyclovir and ganciclovir. These sulfur containing compounds are unique because they do not require phosphorylation in order to inhibit HIV-1 reverse transcriptase (RT). However, these compounds are ineffective against the corresponding HIV-2 enzyme. This is a serious drawback inasmuch as HIV-2 infection is widespread in West African countries and has spread to other areas. See De Cock et al., AIDS, 3(supp.1):S89-S95 (1989).

More recently, Baba et al. (Proc. Natl. Acad. Sci. USA., 88:2356 (1991)) showed that 1-benzyloxy-methyl-5-ethyl-6-(phenylthio)uracil derivatives inhibited HIV-1 replication and HIV-1 RT suggesting that the presence of an acyclic side chain with an intact free hydroxy function is not necessary for antiviral activity.

SUMMARY OF THE INVENTION

The present invention is directed to acyclic 6-phenylselenenyl pyrimidine nucleosides. The general structure of these compounds is depicted below:



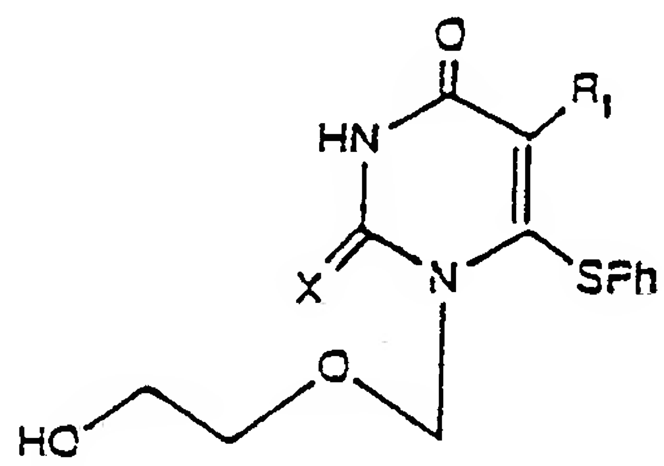
wherein R_1 is hydrogen, halogen, vinyl, halovinyl or C_1-C_3 alkyl, haloalkyl or hydroxyalkyl, R_2 is NH or oxygen, R_3 is oxygen, sulfur or methyl, X is oxygen or sulfur and Y is hydroxyl or hydrogen.

The invention also comprehends antiviral pharmaceutical compositions comprising the novel phenylselenenyl compounds as active ingredients together with conventional pharmaceutical carriers, vehicles or excipients, and methods of antiviral treatment utilizing said compositions.

DETAILED DESCRIPTION OF THE INVENTION

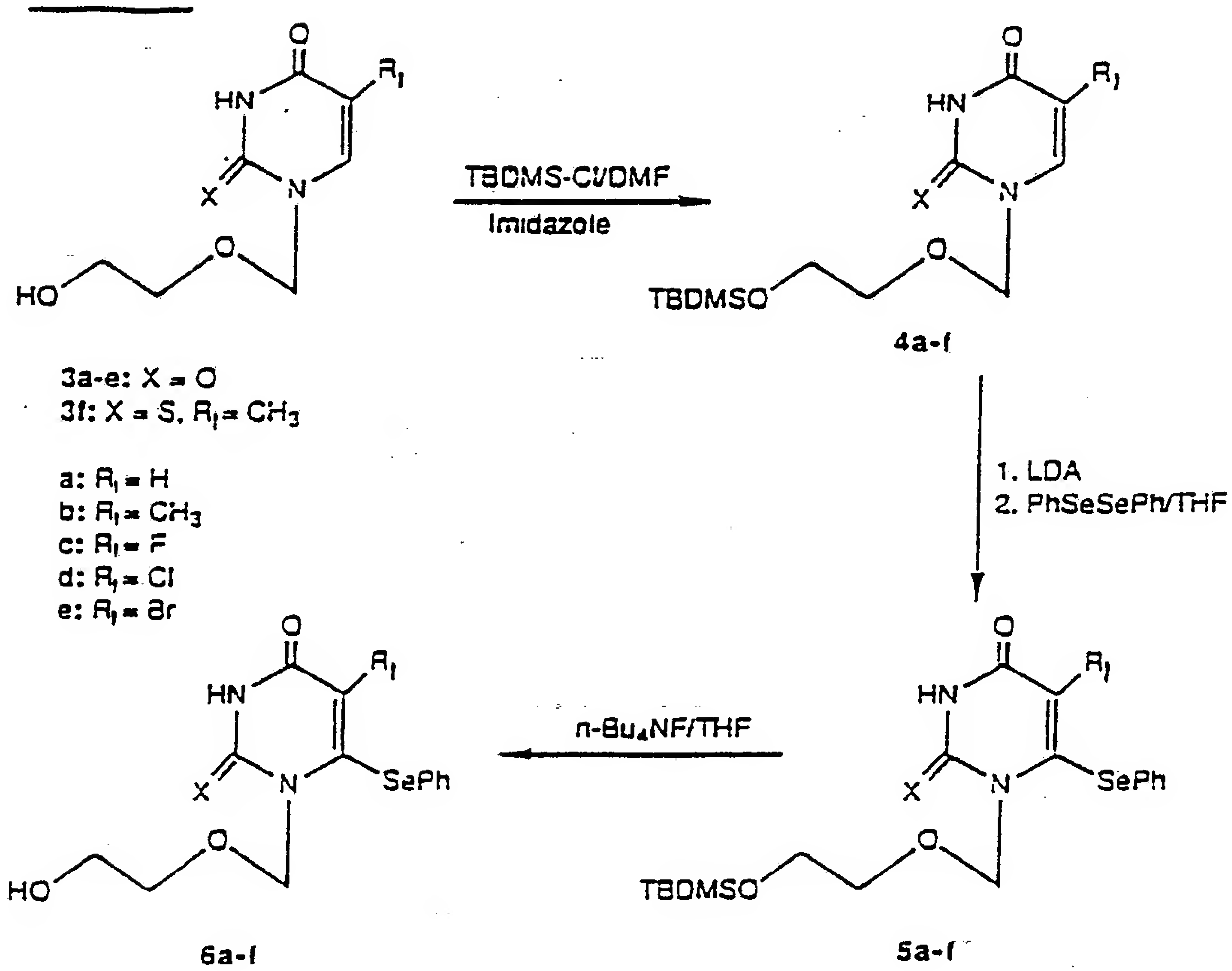
The structures of the prior art HEPT-1 and HEPT-S 2 acyclic nucleosides are shown below, as is a scheme for the synthesis of six species of the novel phenylselenenyl compounds, denoted 6a-f:

HEPT



1: X = O
2: X = S

Scheme I



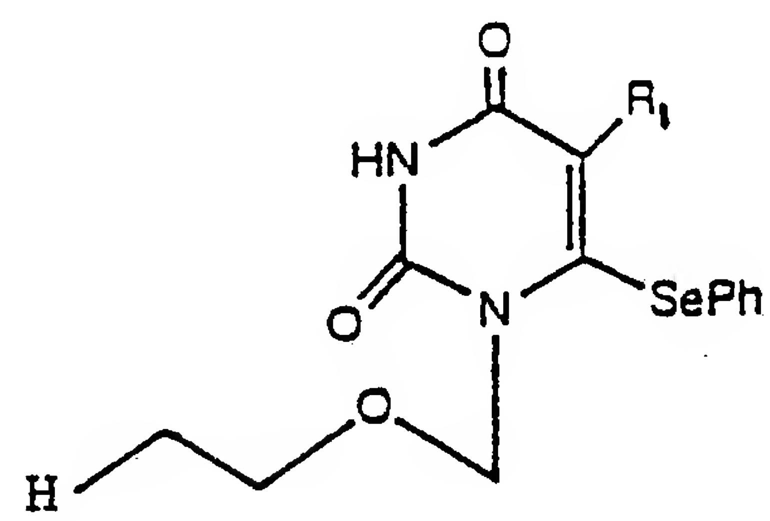
Cosford and Schinazi (J. Org. Chem., 56:2161 (1991)) recently reported on the use of selenium nucleophiles in the synthesis of nucleosides of biological importance. Reich and coworkers (J. Am. Chem. Soc., 97:5434 (1975)) have shown that selenium electrophile, such as diphenyl diselenide, can be used in reactions with unsaturated esters and enolates. This method was adapted to introduce the electrophilic selenium species in pyrimidine acyclic nucleosides by activation of the 6-position with organolithium.

Using the method of Rosowsky et al. (J. Med. Chem., 24:1177 (1981)), 1-[(2-hydroxyethoxy)methyl]uracil 3a and its substituted analogueues 3b-f were prepared via coupling a bis(trimethylsilyl)uracil derivative with a 2-acetoxyethylmethyl ether in the presence of 0.5 molar equivalent of stannic chloride, followed by hydrolysis with sodium methoxide. The hydroxyl group of the acyclonucleosides 3a-f was protected by reaction with tert-butyldimethylsilyl (TBDMS) chloride in DMF in the presence of imidazole (Scheme 1). The TBDMS analogueues 4a-f were obtained as crystalline compounds in almost quantitative yield.

Reaction of 1-[[2-[(tert-butyldimethylsilyl)oxy]ethoxy]methyl]uracil 4a and its derivatives 4b-f in THF with LDA (2.5 equivalent) at -78°C generated regiospecifically the C-6 lithiated species, which was subsequently reacted with diphenyl diselenide. Quenching the reaction mixture with glacial AcOH, followed by silica gel column chromatography, resulted in the

isolation of the corresponding 6-phenylselenenyl analogues 5a-f. Deprotection of the TBDMS group from 5a-f under mild conditions with tetra n-butylammonium flouride afforded the corresponding 1-[(hydroxyethoxy)methyl]-6-(phenylselenenyl)uracil analogues 6a-f in excellent yield (50-70%).

Also included in the present invention are non-hydroxylated analogues of the compounds 6a-f, i.e., 1-ethoxymethyl-6-(phenylselenenyl) pyrimidine nucleosides. Such compounds are exemplified by the following structural formula:



7a-R_1=H
7b-R_1=CH_3

The 1-(ethoxymethyl) pyrimidine precursors of these compounds are prepared by condensing bis(trimethylsilyl) uracil derivatives with chloromethyl ether in the presence of stannic chloride in dichloromethane at room temperature overnight, pouring the reaction mixture into a mixture of cold saturated aqueous sodium bicarbonate solution and trichloromethane, and then separating the resulting emulsion by filtration, with the organic fraction dried and evaporated. Trituration of the oily residue with ether yields the 1-(ethoxymethyl) pyrimidines.

The synthesis of the phenylselenenyl derivatives of the 1-(ethoxymethyl) pyrimidines is accomplished by the same procedure as described previously for the 1-[(2-hydroxy-ethoxy)methyl] pyrimidines.

The present invention also contemplates antiviral pharmaceutical compositions for the oral delivery of an acyclic 6-phenylselenenyl nucleoside compound, exemplified by compounds 6a-f in Scheme 1, to a patient. Such compositions include an antivirally effective amount of one or more of the novel compounds as active ingredients in a pharmaceutically acceptable oral dosage vehicle or form, wherein said vehicle contains inert ingredients that do not interfere with the antiviral activity of said compound.

Dosage forms for oral delivery may include conventional tablets, coated tablets, capsules, caplets, lozenges, liquids, elixirs or any other oral dosage form conventionally used in the pharmaceutical arts.

As pharmaceutically acceptable inert ingredients there are contemplated carriers, excipients, fillers, binders, solvents, etc. which do not interfere with the anti-viral activity of the active ingredient.

In addition, other pharmaceutical agents such as different antivirals or other medicaments may be included in the dosage form of the present invention. Exemplary of suitable additional antiviral compounds are dideoxycytidine, dideoxyinosine, azidothymidine and acyclovir.

Also, fillers such as clays or siliceous earth may be utilized if desired to adjust the size of the dosage form.

Further ingredients such as excipients and carriers may be necessary to impart the desired physical properties of the dosage form. Such physical properties are, for example, release rate, texture and size. Examples of excipients and carriers useful in oral dosage forms are waxes such as beeswax, castor wax, glycowax and carnauba wax, cellulose compounds such as methylcellulose, ethylcellulose, carboxymethylcellulose, cellulose acetate phthalate, hydroxypropylcellulose and hydroxypropylmethylcellulose, polyvinyl chloride, polyvinyl pyrrolidone, stearyl alcohol, glycerin monstearate, methacrylate compounds such as polymethacrylate, methyl methacrylate and ethylene glycol dimethacrylate, polyethylene glycol and hydrophilic gums.

A preferred embodiment of the pharmaceutical compositions of the present invention involves pharmaceutical dosage forms wherein the phenylselenenyl-substituted active ingredient is present in an amount between about 25 and about 1000 mg per dosage unit. The exact dosage administered to each patient will be a function of the physical characteristics of that patient such as body weight.

The invention also comprehends methods of providing antiviral treatment, particularly anti-HIV-1 or HIV-2 treatment, to a patient in need of such treatment consisting of the oral administration to the patient of a pharmaceutical composition

containing at least one of the novel compounds, preferably from about 25 to about 1000 mg of each such compound, from one to four times daily.

The Examples set forth below provide detailed illustrations of the compounds, compositions and methods of the present invention, as well as precursors and intermediates useful for preparation of the novel compounds and synthetic procedures. The Examples are not intended, however, to limit or restrict the scope of the invention in any way, and should not be construed as providing starting materials, reagents, conditions, equipment or reaction parameters which must be utilized exclusively to practice the present invention.

The melting points set forth in the Examples were determined on an Electrothermal IA 8100 digital melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a General Electric QE-300 (300 MHz) spectrometer. Experiments were monitored using TLC analysis performed on Kodak Chromatogram sheets precoated with silica gel and a fluorescent indicator, while column chromatography, employing silica gel (60-200 mesh; Fisher Scientific, Fairlawn, New Jersey) was used for the purification of products. Tetrahydrofuran was freshly distilled from the sodium benzophenone salt. LDA (2.0 M) and diphenyl diselenide were purchased from Aldrich Chemical Company (Milwaukee, Wisconsin). Acyclic pyrimidine nucleosides 3a-f were prepared according to the procedure of Rosowsky et al. Microanalyses were performed at Atlantic Microlabs, Atlanta, GA.

EXAMPLE 1

General Procedure for the Protection of 4a-f. A mixture of the acyclic nucleoside (5 mmol), DMF (20 ml), imidazole (410 mg, 6 mmol) and *tert*-butyldimethylsilyl (TBDMS) chloride (905 mg, 6 mmol) was stirred under argon atmosphere overnight at room temperature. The reaction mixture was poured into water (100 ml), and the precipitate filtered. The resulting solid was dissolved in $CHCl_3$ (80 ml), washed with saturated aqueous $NaHCO_3$ (2 x 40 ml), and H_2O (50 ml), dried over Na_2SO_4, and concentrated in *vacuo* to give the desired TBDMS derivative.

EXAMPLE 2

1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]uracil (4a). Yield 62%, ^{1}HNMR ($CDCl_3$) δ 0.03 (s, 6H, Me_2Si), 0.88 (s, 9H, Me_3C), 3.60-3.78 (A_2B_2, 4H, $SiOCH_2CH_2O$), 5.20 (s, 2H, NCH_2O), 5.75 (d, J = 9 Hz, 1H, 5-H), 7.34 (d, J = 9 Hz, 1H, 6-H), 9.20 (s, 1H, NH, D_2O exchangeable).

EXAMPLE 3

1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]thymine (4b). Yield 89%, ^{1}H NMR ($CDCl_3$) δ 0.03 (s, 6H, Me_2Si), 0.87 (s, 9H, Me_3C), 1.92 (s, 3H, 5-Me), 3.58-3.77 (A_2B_2, 4H, $SiOCH_2CH_2O$), 5.19 (s, 2H, NCH_2O), 7.13 (s, 1H, 6-H), 9.05 (s, 1H, NH, D_2O exchangeable). This compound has been previously reported by Tanaka et al.[4e]

EXAMPLE 4

1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]-5-fluorouracil (4c). Yield 78%, ^{1}H NMR ($CDCl_3$) δ 0.03 (s, 6H, Me_2Si), 0.90 (s, 9H, Me_3C), 3.62-3.78 (A_2B_2, 4H, $SiOCH_2CH_2O$), 5.15 (s, 2H, NCH_2O), 7.42 (d, J = 5 Hz, 1H, 6H), 9.11 (s,1H, NH, D_2O exchangeable).

EXAMPLE 5

1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]-5-chlorouracil (4d). Yield 85%, ^{1}H NMR ($CDCl_3$) δ 0.16 (s, 6H, Me_2Si), 0.89 (s, 9H, Me_3C), 3.61-3.75 (A_2B_2, 4H, $SiOCH_2CH_2O$), 5.20 (s, 2H, NCH_2O), 7.58 (s, 1H, C-6 H), 9.06 (brs, 1H, NH, D_2O exchangeable).

EXAMPLE 6

1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]-5-bromouracil (4e). Yield 83%, ^{1}H NMR ($CDCl_3$) δ 0.06 (s, 6H, Me_2Si), 0.88 (s, 9H, Me_3C), 3.63-3.79 (A_2B_2, 4H, $SiOCH_2CH_2O$), 5.23 (s, 2H, NCH_2O), 7.64 (s, 1H, C-6 H), 9.05 (brs, 1H, NH, D_2O exchangeable).

EXAMPLE 7

1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]-2-thiothymine (4f). Yield 86%, ^{1}H NMR ($CDCl_3$) δ 0.05 (s, 6H, Me_2Si), 0.89 (s, 9H, Me_3C), 1.97 (s, 3H, 5-Me), 3.66-3.80 (A_2B_2, 4H, $SiOCH_2CH_2O$), 5.63 (s, 2H, NCH_2O), 7.33 (s, 1H, 6-H), 9.92 (s, 1H, NH, D_2O exchangeable).

EXAMPLE 8

General Procedure for the Preparation of 6-(Phenylselenenyl)-1-[2-[(*tert*-butyldimethylsilyl)oxy]ethoxy]methyl]thymine Derivatives. To a solution of protected acyclonucleoside (2 mmol) in dry THF (10 ml) at -78°C was added LDA (2.0 M, 2.5 ml, 5 mmol) dropwise over 5 min with stirring under argon atmosphere. The mixture was stirred for 1 h maintaining the temperature below -70°C. A solution of diphenyl diselenide (1.25 g, 4 mmol in 10 ml of THF) was added dropwise over 10 min to the resulting solution, and the mixture was stirred for 30 min at -78°C. The reaction mixture was quenched with AcOH (0.5 ml), and then allowed to warm to room temperature. The solution was concentrated to dryness in *vacuo*, and the residue was purified by silica gel column chromatography to give the corresponding 6-phenylselenenyl derivative.

EXAMPLE 9

1-[[(2-*tert*-Butyldimethylsilyloxy)ethoxy]methyl]-6-(phenylselenenyl)uracil (5a). Purified by column chromatography using $CHCl_3$. Yield 80%; m.p. 138- 140°C; ^{1}H NMR ($CDCl_3$) δ 0.08 (s, 6H, Me_2Si), 0.90 (s, 9H, Me_3C), 3.66-3.82 (A_2B_2, 4H, $SiOCH_2CH_2O$), 5.23 (s, 1H, 5-H), 5.56 (s, 2H, NCH_2O), 7.40-7.65 (m, 5H, SePh), 9.04 (s, 1H, NH, D_2O exchangeable).

EXAMPLE 10

1-[[(2-*tert*-Butyldimethylsilyloxy)ethoxy]methyl]-6-(phenylselenenyl)thymine (5b). Purified by column chromatography using $CHCl_3$. Yield 82%; m.p. 68-72°C; ^1H NMR ($CDCl_3$) δ 0.04 (s, 6H, Me_2Si), 0.86 (s, 9H, Me_3C), 1.95 (s, 3H, 5-Me), 3.59-3.74 (A_2B_2, 4H, $SiOCH_2CH_2O$), 5.68 (s, 2H, NCH_2O), 7.23-7.36 (m, 5H, SePh), 9.02 (s, 1H, NH, D_2O exchangeable).

EXAMPLE 11

1-[[(2-*tert*-Butyldimethylsilyloxy)ethoxy]methyl]-6-(phenylselenenyl)-5-fluorouracil (5c). Purified by column chromatography using $CHCl_3$:MeOH (98:2). Yield 85%; m.p. 125-128°C; ^1H NMR ($CDCl_3$) δ 0.18 (s, 6H, Me_2Si), 0.88 (s, 9H, Me_3C), 3.60-3.76 (A_2B_2, 4H, $SiOCH_2CH_2O$), 5.66 (s, 2H, NCH_2O), 7.26-7.62 (m, 5H, SePh), 9.37 (s,1H, NH, D_2O exchangeable).

EXAMPLE 12

1-[[(2-*tert*-Butyldimethylsilyloxy)ethoxy]methyl]-6-(phenylselenenyl)-5-chlorouracil (5d). Purified by column chromatography using $CHCl_3$:MeOH (98:2). Yield 76%; m.p. 115-118°C; ^1H NMR ($CDCl_3$) δ 0.26 (s, 6H, Me_2Si), 0.92 (s, 9H, Me_3C), 3.63-3.78 (A_2B_2, 4H, $SiOCH_2CH_2O$), 5.73 (s, 2H, NCH_2O), 7.28-7.50 (m, 5H, SePh), 9.12 (brs; 1H, NH, D_2O exchangeable).

EXAMPLE 13

1-[[(2-*tert*-Butyldimethylsilyloxy)ethoxy]methyl]-6-(phenylselenenyl)-5-bromouracil (5e). Purified by column chromatography using $CHCl_3$:MeOH (95:5). Yield 65%; m.p. 102-106°C; ^1H NMR ($CDCl_3$) δ 0.02 (s, 6H, Me_2Si), 0.86 (s, 9H, Me_3C), 3.62-3.75 (A_2B_2, 4H, $SiOCH_2CH_2O$), 5.73 (s, 2H, NCH_2O), 7.28-7.47 (m, 5H, SePh), 8.79 (s, 1H, NH, D_2O exchangeable).

EXAMPLE 14

1-[[(2-*tert*-Butyldimethylsilyloxy)ethoxy]methyl]-6-(phenylselenenyl)-2-thiothymine (5f). Purified by column chromatography using $CHCl_3$:MeOH (98:2). Yield 77%; m.p. 97-100°C; 1H NMR ($CDCl_3$) δ 0.03 (s, 6H, Me_2Si), 0.86 (s, 9H, Me_3C), 1.88 (s, 3H, 5-Me), 3.71 (s, 4H, $SiOCH_2CH_2O$), 5.79 (s, 2H, NCH_2O), 7.21-7.35 (m, 5H, SePh), 9.73 (s, 1H, NH, D_2O exchangeable).

EXAMPLE 15

General Procedure for Deprotection of the *tert*-Butyldimethyl-silyl (TBDMS) Group. To the protected derivative (1 mmol) dissolved in THF (5 ml), was added a solution of tetra-*n*-butylammonium fluoride (1.2 mmol in 2 ml of THF). The resulting reaction mixture was stirred for 30 min at room temperature, and evaporated to dryness. The residue was purified by silica gel column chromatography and crystallized from a suitable solvent.

EXAMPLE 16

1-[(2-Hydroxyethoxy)methyl]-6-(phenylselenenyl)uracil (6a). Purified by column chromatography using $CHCl_3$:MeOH (98:2). Yield 86%; m.p. 157-159°C (toluene); ^1H NMR ($CDCl_3$) δ 2.23 (s, 1H, OH, D_2O exchangeable), 3.70-3.79 (m, 4H, OCH_2CH_2O), 5.23 (s, 1H, 5-H), 5.78 (s, 2H, NCH_2O), 7.39-7.68 (m, 5H, SePh), 9.30 (s, 1H, NH, D_2O exchangeable). Anal. ($C_{13}H_{14}N_2O_4Se$) C, H, N.

EXAMPLE 17

1-[(2-Hydroxyethoxy)methyl]-6-(phenylselenenyl)thymine (6b). Purified by column chromatography using $CHCl_3$:MeOH (98:2). Yield 75%; m.p. 108-109°C (toluene); ^1H NMR ($CDCl_3$) δ 1.75 (s, 1H, OH, D_2O exchangeable), 2.03 (s, 3H, 5-Me), 3.65 (m, 4H, OCH_2CH_2O), 5.67 (s, 2H, NCH_2O), 7.26-7.38 (m, 5H, SePh), 9.32 (s, 1H, NH, D_2O exchangeable). Anal. ($C_{14}H_{16}N_2O_4Se$) C, H, N.

EXAMPLE 18

1-[(2-Hydroxyethoxy)methyl]-6-(phenylselenenyl)-5-fluorouracil (6c). Purified by column chromatography using $CHCl_3$:MeOH (95:5). Yield 83%; m.p. 130-132°C (EtOAc); 1H NMR (DMSO-d_6) δ 3.37-3.49 (m, 4H, OCH_2CH_2O), 4.65 (t, 1H, OH, D_2O exchangeable), 5.45 (s, 2H, NCH_2O), 7.27-7.63 (m, 5H, SePh), 11.96 (s, 1H, NH, D_2O exchangeable). Anal. ($C_{13}H_{13}FN_2O_4Se$) C, H, N.

EXAMPLE 19

1-[(2-Hydroxyethoxy)methyl]-6-(phenylselenenyl)-5-chlorouracil (6d). Purified by column chromatography using $CHCl_3$:MeOH (95:5). Yield 68%; m.p. 142-143°C (EtOAc); 1H NMR (DMSO-d_6) δ 3.38-3.52 (m, 4H, OCH_2CH_2O), 4.60 (t, 1H, OH, D_2O exchangeable), 5.53 (s, 2H, NCH_2O), 7.28-7.53 (m, 5H, SePh), 12.04 (s, 1H, NH, D_2O exchangeable). Anal. ($C_{13}H_{13}ClN_2O_4Se$) C, H, N.

EXAMPLE 20

1-[(2-Hydroxyethoxy)methyl]-6-(phenylselenenyl)-5-bromouracil (6e). Purified by column chromatography using $CHCl_3$:MeOH (90:10). Yield 79%; m.p. 144-145°C $CHCl_3$); 1H NMR (DMSO-d_6) δ 3.27-3.51 (m, 4H, OCH_2CH_2O), 4.59 (s, 1H, OH, D_2O exchangeable), 5.53 (s, 2H, NCH_2O), 7.24-7.51 (m, 5H, SePh), 12.00 (s, 1H, NH, D_2O exchangeable). Anal. ($C_{13}H_{13}BrN_2O_4Se$) C, H, N.

EXAMPLE 21

1-[(2-Hydroxyethoxy)methyl]-6-(phenylselenenyl)-2-thiothymine (6f). Purified by column chromatography using $CHCl_3$:MeOH (95:5). Yield 83%; m.p. 108-109°C (toluene); 1H NMR ($CDCl_3$) δ 1.64 (s, 1H, OH, D_2O exchangeable), 1.93 (s, 3H, 5-Me), 3.67-3.75 (m, 4H, OCH_2CH_2O), 6.27 (s, 2H, NCH_2O), 7.26-7.35 (m, 5H, SePh), 10.01 (s, 1H, NH, D_2O exchangeable). Anal. ($C_{13}H_{13}N_2O_4SSe$) C, H, N.

EXAMPLE 22

General Procedure for the Preparation of 1-(Ethoxymethyl)pyrimidines. Chloromethyl ethyl ether was condensed with the bis(trimethylsilyl)uracil derivatives in the presence of one molar equivalent of stannic chloride in CH_2Cl_2 at room temperature for overnight. The reaction mixture poured slowly into a mixture of cold saturated aqueous $NaHCO_3$ solution and $CHCl_3$. The resulting emulsion was separated by filtration through Celite, the organic fraction dried and evaporated. Trituration of the oily residue with ether afforded the product as colorless crystals.

EXAMPLE 23

1-(Ethoxymethyl)uracil. Yield 42%; m.p. 124-126°C; ^1H NMR ($CDCl_3$) δ 1.21 (t, J= 7 Hz, 3H, CH_3CH_2O), 3.59 (q, 2H, J = 7.2 Hz, CH_3CH_2O), 5.20 (s, 2H, NCH_2O), 5.86 (d, J = 9 Hz, 1H, 5-H), 7.43 (d, J = 9 Hz, 1H, 6-H), 9.68 (s, 1H, NH, D_2O exchangeable).

EXAMPLE 24

1-(Ethoxymethyl)thymine. Yield 54%; m.p. 104-106°C; ^1H NMR ($CDCl_3$) δ 1.20 (t, J= 6.8 Hz, 3H, CH_3CH_2O), 1.93 (s, 3H, 5-Me), 3.58 (q, J= 6.8 Hz, 2H, CH_3CH_2O), 5.22 (s, 2H, NCH_2O), 7.14 (s, 1H, 6-H), 9.26 (s, 1H, NH, D_2O exchangeable).

EXAMPLE 25

General Procedure for the Preparation of 1-Ethoxymethyl-6-(phenylselenenyl) pyrimidines. Synthesis of phenylselenenyl derivatives was identical to that used for the preparation of 1-[(hydroxyethoxy)methyl-6-(phenylselenenyl) analogues.

EXAMPLE 26

1-Ethoxymethyl-6-(phenylselenenyl)uracil (7a) Purified by column chromatography using $CHCl_3$:MeOH (98:2). Yield 83%; m.p. 176-178°C (ethanol); ^1H NMR ($CDCl_3$) δ 1.26 (t, J= 6.8 Hz, 3H, CH_3CH_2O), 3.65 (q, 2H, J = 6.8 Hz, CH_3CH_2O), 5.28 (d, J = 11.2 Hz, 1H, 5-H), 5.52 (s, 2H, NCH_2O), 7.38-7.66 (m, 5H, SePh), 9.23 (s, 1H, NH, D_2O exchangeable). Anal. ($C_{13}H_{14}N_2O_3Se$) C, H, N.

EXAMPLE 27

1-Ethoxymethyl-6-(phenylselenenyl)thymine (7b) Purified by column chromatography using $CHCl_3$:MeOH (98:2). Yield 85%; m.p. 134-135°C (ethanol); ^1H NMR ($CDCl_3$) δ 1.17 (t, J= 6.5 Hz, 3H, CH_3CH_2O), 1.98 (s, 3H, 5-Me), 3.57 (q, J= 6.5 Hz, 2H, CH_3CH_2O), 5.63 (s, 2H, NCH_2O), 7.25-7.37 (m, 5H, SePh), 9.35 (s, 1H, NH, D_2O exchangeable). Anal. ($C_{14}H_{16}N_2O_3Se$) C, H, N.

EXAMPLE 28

Antiviral Evaluations

Compounds 3a-f were evaluated in human peripheral blood mononuclear (PBM) cells infected with HIV-1 (strain LAV). Virus yield was determined by measuring the level of HIV-1 RT present in disrupted virions obtained from supernatant from cells exposed to the drugs. The results are set forth in Table 1. None of these compounds inhibited HIV-1 replication at concentrations greater than 100 μM. The 5-halogeno derivatives (3c-3e) were found to have minimal toxicity to uninfected PBM cells. Introduction of a phenylselenenyl moiety at the 6-position of these acyclic nucleosides produced compounds (6a-f) which were as potent against HIV-1 as HEPT, the lead compound (see Table 1). The median effective concentration (EC_{50}) for these compounds ranged from 0.96 to 13.0 μM. The results obtained with HEPT in human PBM cells were similar to those reported by Miyasaka et al., J. Med. Chem., 32:2507 (1989), in MT-4 cells (EC_{50}=5.3 μM versus 7.0 μM). The uracil analogue (6a) appeared to be less effective than the 5-substituted compounds. Whereas the thymine analogue (6b) exhibited no cytotoxicity in human PBM or in Vero cells, some cytotoxicity was noted with the corresponding halogeno derivatives (6c-e) in Vero cells. This suggests that some of the selenium analogues may exhibit toxicity in other rapidly dividing cells such as human bone marrow cells. When tested in human PBM cells infected with HIV-2 (strain ROD-2), compounds 6a-f were found to have activity similar to that

obtained with HIV-1, with the exception of the (6-phenylselenenyl)-thymine derivative (6b) which was about 25-fold less active. As previously reported by Baba et al., Biochem. Biophys. Res. Commun., 165:1375 (1989), HEPT was also not active against HIV-2 in this cell culture system. The selectivity of the novel compounds appears to be directed at HIV-1 and HIV-2, since none of the selenium containing compounds were effective against HSV-1 in a plaque reduction assay in Vero cells (see Schinazi et al., Antimicrob. Agent. Chemother., 22:499 (1982)).

The effect of the 6-phenylselenenyl analogues (6a-f) on HIV-1 RT in a cell free system was also determined since HEPT had been reported to inhibit HIV-1 RT. Surprisingly, none of the compounds exhibited marked anti-RT activity when tested up to 100 μM. Whereas HEPT had an IC_{50} of 17.5 μM, the thymine analogue 6a had an IC_{50} of 90.3 μM. None of the compounds evaluated inhibited DNA polymerase α purified from human PBM cells when tested up to a concentration of 100 μM.

The novel selenenyl compounds were unexpectedly found to have selective antiviral activity against both HIV-1 and HIV-2 in primary human lymphocytes, and are not inhibitors of HIV-1 RT. Taken together, the results suggest that the activity of the selenium analogues is produced by inhibition of a viral target which is different from the corresponding sulfur containing analogues, such as HEPT.

Table 1. Biological Evaluation of Various Substituted Acyclic Pyrimidine Nucleosides.

Code	1-[2-Hydroxyethoxymethyl]-analogue of	Anti-HIV-1 in PBMC EC_{50}, μM	Anti-HIV-2 in PBMC EC_{50}, μM	Toxicity in PBMC IC_{50}, μM	HIV-1 RT[a] inhibition IC_{50}, μM	DNA pol. α derived from PBMC IC_{50}, μM	Toxicity in Vero cells IC_{50}, μM	Anti-HSV-1 in Vero cells EC_{50}, μM
3a	Uracil	> 100	ND	> 100	ND	ND	> 100	> 100
3b	Thymine	> 100	ND	> 100	ND	ND	> 100	≥ 100
3c	5-Fluorouracil	> 100	ND	67.0	ND	ND	> 100	> 100
3d	5-Chlorouracil	> 100	ND	ND	ND	ND	> 100	> 100
3e	5-Bromouracil	> 100	ND	100	ND	ND	> 100	≥ 100
3f	2-Thiothymine	> 100	ND	> 100	ND	ND	> 100	> 100
1	6-(Phenylthio)thymine (HEPT)	5.30	> 100	≥ 100	17.5	> 100	> 100	> 100
6a	6-(Phenylselenenyl)uracil	13.0	9.6	> 100	139	> 100	> 100	> 200
6b	6-(Phenylselenenyl)thymine	0.96	25.6	> 200	90.3	> 100	> 100	> 100
6c	6-(Phenylselenenyl)-5-fluorouracil	2.0	9.1	> 100	> 100	> 100	25.7	> 100
6d	6-(Phenylselenenyl)-5-chlorouracil	3.1	7.9	> 100	> 100	> 100	7.5	> 100
6e	6-(Phenylselenenyl)-5-bromouracil	3.7	2.0	≥ 100	> 100	> 100	8.2	> 100
6f	6-(Phenylselenenyl)-2-thiothymine	2.8	5.8	> 100	> 100	> 100	60.7	> 100
	Guanosine (ACV as control)	> 50	ND	> 100	ND	ND	> 100	0.03
	3'-Azido-3'-deoxythymidine (AZT or AZT-TP as control)	0.004	≤ 0.002	> 100	< 0.1	> 100	26.0	> 100

The non-hydroxylated (i.e., 1-ethoxymethyl) analogues 7a and 7b were also evaluated for anti-HIV 1 activity and toxicity in PBM and Vero cells in accordance with the procedures outlined above. The results of these tests are set forth in Table 2.

Table 2

Compound	Anti-Hiv-1: PBM cells EC_{50}, µM	Cytoxicity: PBM cells IC_{50} µM	Cytoxicity: Vero cells IC_{50}, µM
1-Ethoxymethyl-6-(phenylselenenyl)uracil	22.8	>100	28.0
1-Ethoxymethyl-6-(phenylselenenyl)thymine	1.2	>100	167.0

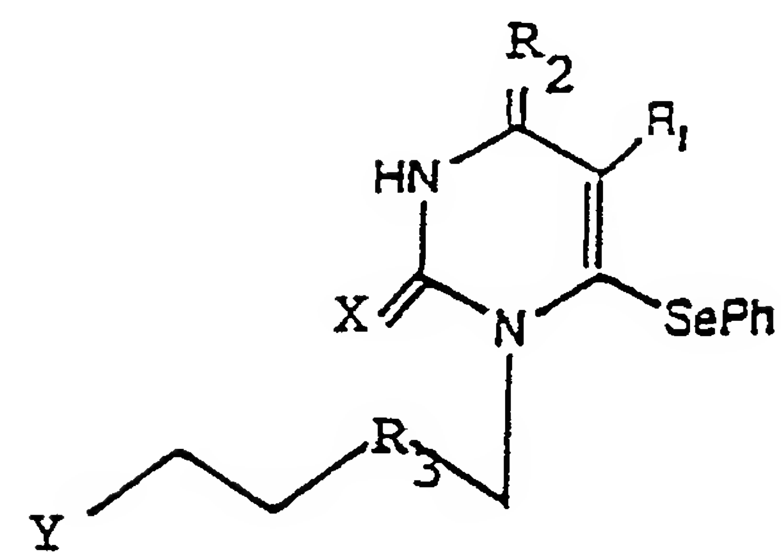
It has thus been shown that there are provided compounds, compositions and methods which achieve the various objects of the invention and which are well adapted to meet the conditions of practical use.

As various possible embodiments might be made of the above invention, and as various changes might be made in the embodiments set forth above, it is to be understood that all matters herein described are to be interpreted as illustrative and not in a limiting sense.

What is claimed as new and desired to be protected by Letters Patent is set forth in the following claims.

I CLAIM:

1. A compound having the formula:



wherein R_1 is hydrogen, halogen, vinyl, halovinyl or C_1-C_3 alkyl, haloalkyl or hydroxyalkyl, R_2 is NH or oxygen, R_3 is oxygen, sulfur or methyl, X is oxygen or sulfur and Y is hydroxyl or hydrogen.

2. A compound according to claim 1 wherein R_1 is hydrogen.

3. A compound according to claim 1 wherein R_1 is methyl.

4. A compound according to claim 1 wherein R_1 is halogen.

5. A compound according to claim 1 wherein X is oxygen.

6. A compound according to claim 1 wherein X is sulfur.

7. A compound according to claim 1 wherein Y is hydroxyl.

8. A compound according to claim 1 wherein Y is hydrogen.

9. 1-[(2-hydroxyethoxy)methyl]-6-(phenylselenenyl) uracil.

10. 1-[(2-hydroxyethoxy)methyl]-6-(phenylselenenyl) thymine.

11. 1-[(2-hydroxyethoxy)methyl]-6-(phenylselenenyl)-5-fluorouracil.

12. 1-[(2-hydroxyethoxy)methyl]-6-(phenylselenenyl)-5-chlorouracil.

13. 1-[(2-hydroxyethoxy)methyl]-6-(phenylselenenyl)-5-bromouracil.

14. 1-[(2-hydroxyethoxy)methyl]-6-(phenylselenenyl)-2-thiothymine.

15. 1-(ethoxymethyl)-6-(phenylselenenyl)uracil.

16. 1-(ethoxymethyl)-6-(phenylselenenyl)thymine.

17. A pharmaceutical composition for oral administration to a patient requiring antiviral treatment comprising an antivirally effective amount of a compound according to claim 1 in a pharmaceutically acceptable oral dosage form.

18. A composition according to claim 17 wherein said dosage form further comprises inert carriers, excipients, fillers, binders or solvents.

19. A composition according to claim 17 wherein said dosage form is a tablet, capsule, caplet, lozenge, liquid or elixir containing from about 25 to about 1000 mg of said compound per dosage unit.

20. A composition according to claim 19 wherein said dosage form is a tablet.

21. A composition according to claim 17 which further comprises an antiviral agent selected from the group consisting of dideoxycytidine, dideoxyinosine, azidothymidine and acyclovir.

22. A method of providing antiviral treatment to a patient infected with a virus consisting of the oral administration to the patient of a pharmaceutical composition in accordance with claim 17 from one to four times daily.

23. A method according to claim 22 wherein said virus is HIV-1.

24. A method according to claim 22 wherein said virus is HIV-2.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/03824

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07C239/06, 239/08; A61K 31/505
US CL :544/302,303,309,311,312,313;5 4/269,274
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO89/09213 (Miyasaka et al.) 10 May 1989. See Abstract and pages 8 through 27.	1-24

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be part of particular relevance
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search	Date of mailing of the international search report
21 AUGUST 1992	07 OCT 1992

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